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Molybdenum effects on muskmelon (Cucumbis melon) seedlings

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

ABA	Abscisic Acid
IAA	Indole-3-Acetic Aid
LAI	Leaf Area Index
LSD	Least Significant Difference
Mo	Molybdenum
MOT_1	Molybdenum Transport Proteins
Moco	Molybdenum Cofactor
NPK	Nitrogen-Phosphorus-Potassium
NO_3	Nitrate
NO_2	Nitrite
RCBD	Randomized Complete Block Design
Redox	Oxidation-Reduction Reactions
ROS	Reactive Oxygen Species

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ABSTRACT

Molybdenum (Mo) is required for the formation of five molybdoenzymes involved in plant growth and development. Once Mo is incorporated into molybdoenzymes, these enzymes can metabolize complex redox reactions, thus allowing plants to utilize nitrate, detoxify sulfites, create carbohydrates, and regulate hormone activity. The high pH and minimal organic matter content in soils from the Sonoran Desert of the southwestern US, increase Mo mobility and availability to crops. Consequently, Mo leaches from the root zone during irrigation. Mo deficiency symptoms are common in muskmelon (*Cucumis melo* L.) desert production mid-way through the growing season. These symptoms often are incorrectly diagnosed as sodium toxicity or nitrate deficiency. If Mo deficiencies are prevented during the initial stages of seedling development, the seedling growth rate would increase, thus widening the planting window for muskmelons producers. After a series of six simple preliminary experiments, a finalized greenhouse experiment was conducted. Two different cultivars of muskmelons, Olympic Gold and Caravelle, were used to determine the Mo concentration required in a foliar application to increase plant growth and development. Four weeks after seeding, true leaf numbers, root length, shoot length, wet weights, dry weights, Mo, nitrate-nitrogen, total nitrogen, carbon, sulfur, zinc, calcium, sodium, and boron concentrations were evaluated. Analysis of variance and linear regression were used to analyze the data. It was concluded that weekly foliar applications of 8-mg/L Mo after germination for four weeks increased seedling growth, and that there was a positive correlation between Mo and sulfur, Mo and zinc, as well as Mo and boron. The addition of

Mo can help farmers reduce input costs by increasing micronutrient uptake and widening the planting window of muskmelons by increasing growth rate.

CHAPTER 1

INTRODUCTION

Background Information

Molybdenum in Soil

The natural source of molybdenum (Mo) in soil is igneous and sedimentary rock that forms the parent material, located just below soil horizon C. Through chemical and physical weathering, Mo follows one of two pathways: (1) the minerals within the parent material are broken down and Mo remains in the soil column and deposited in horizons O, A, B, C or (2) Mo is translocated to other regions as alluvial, loess, or glacial deposits (Smith et al., 1997). The three-main natural mineral deposits of raw materials that naturally contain Mo in soil are molybdenite, wulfenite, and ferrimolybdenite. Through chemical weathering these minerals break down, releasing soluble molybdate (MoO_4^-) for immediate plant uptake (Kaiser et al., 2005).

Like most nutrients, the availability of Mo for plant uptake is highly dependent upon plant growing environment. The environmental factors that affect Mo root absorption are: soil pH, soil organic matter content, soil texture, water drainage, and concentration of other nutrients (mainly oxides such as iron, aluminum, manganese), present in the soil (Haque, 1987; Kaiser et al., 2005; Brady et al., 2008). These environmental factors interact and overlap, and as the chemistry of the soil changes, Mo adsorbs onto cations in the soil during electro-chemical interactions with metals, salts, clay, dissolved organic compounds, and carbohydrates (Kaiser et al., 2005).

Molybdenum and Soil pH

Like other essential nutrients for plant growth and development, Mo availability is pH-dependent. In fact, for every unit increase in soil pH, Mo availability within the soil increases one-hundred-fold (Gupta, 1997). Mo is classified as a micronutrient; however, the inverse relationship between Mo availability and soil pH is opposite to most other micronutrients. Alkaline soil pH allows Mo to adsorb readily to cations such as calcium, sodium, potassium, and magnesium. This process increases the available Mo concentrations for immediate plant uptake and Mo losses through leaching (Kaiser et al, 2005). Under acidic soil conditions, the opposite occurs. Mo forms a chemical bond with silicates, iron oxide, aluminum oxide, and manganese oxidize to help Mo remain in the soil profile. These complexes prevent root uptake and increase Mo deficiencies in higher level plants (Kaiser et al, 2005; International Plant Nutrition Institute, 2017).

Molybdenum and Other Soil Environmental Factors

Soil moisture also affects Mo availability within the soil profile. Heavy textured soils and soils high in organic matter drain slowly (Kaiser et al., 2005). When these factors are present, Mo accumulates in the soil and binds with the clay while organic matter slowly decomposes (Kaiser et al., 2005). Molybdenum also binds with iron and other oxides within the organic matter and clay, thus reducing the availability of Mo for root uptake. Once the soil organic matter breaks down in the soil profile, Mo can mineralize and become available for plant uptake (Haque, 1987).

Molybdenum in Plants

Molybdenum and Enzyme Activity

Plants require very small quantities of Mo, compared to the 18 essential nutrients required for plant growth and development. An example of this is evident in comparison of Mo to other major and secondary nutrients, such as nitrogen. The adequate range of total nitrogen in muskmelon leaf tissue at the first growth stage (early runner) is 45.0-55.0 g/kg (Bryson, 2014), whereas Mo concentration in healthy leaf tissue is just above 0.5 mg/kg or 0.0005 g/kg (Gu1ber, 1982). Although the required quantity of Mo is minimal, its metabolic role is substantial. Without Mo plants would perish.

Extensive research over the last 15 years has broadened our scientific understanding of the role of Mo in higher plants. Mo research in higher level plants has evolved from concepts learned in prokaryotes, such as bacterium, fungi, and unicellular algae (Mendel et al., 1999). Mo is part of over sixty different enzymes (Datta et al., 2011), known as molybdoenzymes. Less than ten types of molybdoenzyme are found in plants (Mendel, 2011). The primary role of molybdoenzymes is to catalyze oxidation-reduction reactions taking place within the carbon, nitrogen, and sulfur cycles of plants (Kisker, 1997; Datta et al., 2011). Consequently, Mo plays a critical role in crop growth and development.

Molybdenum uptake - from the soil into the plant root

The mode of Mo transport and absorption in higher plants is still under scientific debate. The two theories under consideration hypothesize that Mo uptake takes place through transporters located within plant roots, either via phosphate-phosphorus transporters (Gupta, 1997; Datta et al., 2011), or sulfate transporters (Mendel, 2011; Broadley et al., 2012).

Phosphorus Theory

Plant available Mo, molybdate, chemically bonds with phosphate-phosphorus to form a phosphomolybdate complex within the soil. The phosphomolybdate uptake through the roots is favored over phosphate-phosphorus ion uptake (Haque, 1987). Phosphomolybdate has greater solubility in water and greater availability in soil solution than phosphate-phosphorus. The greater solubility enhances differential adsorption and requires less plant energy for nutrient uptake through the root system. Other researchers concluded that Mo uptake also may occur through the plasma membrane of plants root cells via phosphorus transporting sites (Gupta, 1997). However, other scientists have reported that Mo uptake is not affected by phosphorus transport sites. When soils contain adequate levels of available phosphate-phosphorus and low Mo, molybdenum uptake still occurs suggesting other modes of Mo transport (Kaiser et al., 2005).

Sulfur Theory

Mo transport proteins, such as MOT_1, belong to the same family as other sulfate carriers. The role of MOT_1 is to actively transport Mo into the roots and through the plant. This movement through the plant is accomplished by cellular membrane transport from cell to cell of the endomembrane system (Mendel, 2011).

The relative molecular size of molybdate to sulfate is very similar (Kaiser et al., 2005). Therefore, researchers speculate that Mo follows the same pathway as sulfate-sulfur. However, not all scientists agree. Past research has shown that sulfate-sulfur can block the uptake of Mo from the soil into the roots. Higher concentrations of sulfate-sulfur out compete Mo for sulfur transporters (Broadley et al., 2012), possibly due to similar molecular size.

From Molybdate to Moco

Once inside the plant, molybdate is not biologically active. In order for Mo to be biologically beneficial to the plant, this anion must be inserted into Mo-dependent enzymes, molybdoenzymes, for plant utilization. For this process to occur, it is essential that molybdate be first chemically transformed into a Mo-cofactor known as Moco. The biosynthesis of Moco is a four-step process which occurs within the cell's cytosol. This process involves multiple proteins, nitrogen, sulfur, phosphorus, copper, and in some cases iron (Schwarz et al., 2006; Mendel, 2011). The chemical products from each biosynthetic step are: cPMP, MPT, adenylated MPT, and Moco; respectively (Mendel, 2011). During the last step of Moco formation, Mo attaches to a specific pterin complex forming molybdopterin (Schwarz et al., 2006; Mendel, 2011).

From Moco to molybdoenzymes

Moco can follow two possible pathways: (1) Moco is transported to the molybdoenzyme and inserted into the molybdoenzyme at the right time/right place; or (2) Moco is attached to a carrier protein for protection, storage and, when required, be transported to a molybdoenzyme (Mendel et al., 1999; Schwarz et al., 2006; Mendel, 2011). The supply and demand for Moco is what ultimately determines which pathway Moco will follow within higher level plants.

Without Moco, metabolic functions, such as nitrogen utilization, in higher level plants would not occur. To complicate matters, Moco alone is very unstable and easily oxidized. Therefore, Moco must bind itself to proteins, specifically carrier proteins. When Moco becomes unstable, it disintegrates. In the disintegration process, Moco first separates from the carrier protein, then the Mo molecule dislodges from Moco and Mo becomes inactive.

Last, the remaining components of Moco oxidize and are permanently rendered useless as Mo transporters (Mendel, 2011). The final Mo anion pathway after it separates from Moco is unknown.

Research on how Moco reaches the molybdoenzymes is incomplete; however, based upon unicellular algae observations, it is hypothesized that Moco-binding proteins lock onto and transport Moco to molybdoenzymes (Schwarz et al., 2006; Mendel, 2011). Once Moco reaches its intended destination, incorporating Moco into molybdoenzymes begins. Unfortunately, the attachment site for Moco is not on the outer surface of the molybdoenzymes, but in the center heart of the molybdoenzymes. Using crystallographic analysis, the timing for the insertion of Moco into the molybdoenzyme takes place either at the very beginning or during the folding process of apoproteins, which are used to form the molybdoenzymes (Mendel, 2011).

The Moco and Moco-binding/transport protein complex contains all the components necessary to attach the Mo molecule to the precise location and alignment within the molybdoenzyme. Although Moco-binding/transport proteins aid in the attachment process (Mendel, 2011), it is the pterin component that positions Mo directly into the molybdoenzyme's activation site (Schwarz et al., 2006). Pterin binds to Mo in the last step of Moco formation, thus forming molybdopterin. Pterin not only delivers and positions the biological activating factor Mo to the molybdoenzyme, but pterin also regulates the activity of Mo within the molybdoenzyme (Schwarz et al., 2006).

General role of molybdenum in molybdoenzymes

Within molybdoenzymes, the purpose of Mo is to serve as the catalytic metal for oxidation-reduction (redox) reactions. Depending upon the functionality of the

molybdoenzyme, Mo will either donate its electrons and become oxidized or accept electrons and have its oxidative state reduced (Schwarz et al., 2006; Mendel, 2011). To help regulate and aid Mo in the redox process, pterin adjusts the pace of the redox chemical reactions as well as assists in electron transfer to and from Mo (Schwarz et al., 2006).

Breakdown of molybdoenzymes. Is Mo recyclable?

Once the redox reaction is complete, it is believed that molybdoenzymes disintegrate and Moco oxidizes as part of the breakdown process (Mendel, 2011), similar to other enzymes. Although not confirmed, it is believed that Mo is not recyclable within the plant. Whether the Mo remains within the plant, exits via the stomata, or photosynthate deposits into the soil is unknown.

Categorizing molybdoenzymes

Within higher level plants, there are five known families of molybdoenzymes: nitrogenase, nitrate reductase, sulfite oxidase, aldehyde oxidase, and xanthine oxidase (Haque, 2008). These families subdivided into two classes, excluding nitrogenase: (1) SO-class, and (2) XO oxidase family, based upon the redox reaction (Kisker et al., 1997; Mendel, 2011). The nitrogenase enzyme is maintained as separate, since it is found in nitrogen-fixing bacteria that form symbiotic relationships with plants in the legume family. Families of molybdoenzymes that affect germination in muskmelons are discussed in more detail below.

Nitrate reductase (SO-class)

The most common form of nitrogen absorbed by plants is nitrate-nitrogen; however, higher level plants cannot utilize nitrate directly. Nitrate must first be reduced by the molybdoenzyme nitrate reductase to form nitrite, and then reduced by a non-molybdoenzyme

nitrite reductase, to form ammonium (Gupta, 1997). For the purpose of this discussion, we will focus on molybdoenzymes.

Nitrate reductase is located only in the cytosol of the plant cells (Mendel, 2011). This enzyme is positioned near chlorophyll molecules inside the chloroplast, for logistical nitrite transfer. It catalyzes a three-step catalytic cycle ending with Mo oxidization (Mendel, 2011). In the redo reaction, Mo donates an electron to nitrate, thus reducing nitrate to nitrite and creating water as a byproduct: $NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O$ (Mendel et al., 1999; Schwarz et al., 2006). As simple as this may appear, nitrate reductase is an essential enzyme for plant health. Not only is nitrate reductase the first step in converting inorganic nitrate into organic nitrogen products, such as future plant proteins and DNA, but nitrate reductase is one of the initial steps in the nitrate assimilation process (Kaiser et al., 2005). Nitrate assimilation is the transformation of inorganic nitrogen into organic forms utilized by plants. Therefore, nitrate assimilation is responsible for internal plant nutrition as well as the way the plants break down and utilize both carbon and nitrogen (Mendel et al., 1999).

The nitrate reductase concentration in plant cells corresponds directly to the concentration of Mo found in plant organelles (Gopal et al., 2017; Nelson, 1984). Plants deficient in Mo continue to uptake nitrate-nitrogen from the soil and store the excess nitrogen within the leaf. When levels of nitrate-nitrogen within the plant tissue are high due to low Mo concentrations, a reduction of both chlorophyll and ascorbic acid may occur (Bryson et al., 2014). Ascorbic acid protects the plant from oxidation damage caused during redox reactions, such as induced hydrogen peroxide formation and DNA breakage (Datta et al., 2011), and allows the plant to adjust to abiotic stresses.

Since nitrate-nitrogen continues to accumulate in the leaf, visual symptoms like chlorosis and leaf burn begin to develop at the leaf margin (Kaiser et al., 2005). This nitrate-nitrogen accumulation also creates false 'adequate total nitrogen' readings in tissue analyses if Mo is over looked, leading to misdiagnosis of the true problem. As a result, plant growth and development will slow down and yield will be lost if the over accumulation of nitrate-nitrogen is not rectified.

Sulfite oxidase (SO-class)

Most of the research dedicated to understanding sulfite oxidase has occurred using chicken livers (Schwarz et al., 2006; Mendel, 2011) because, in the Kingdom Animalia, SO detoxifies the body preventing premature death in humans (Kappler et al., 2014). However, within the last twelve years, scientists have learned that there are significant differences between sulfite oxidase in the kingdom Animalia versus the kingdom Plantae; scientists still are making headway. Sulfite oxidase in chicken liver resides in the mitochondria. However, in higher level plants sulfite oxidases are found inside peroxisomes within the cytoplasm of plant cells, not in the chloroplasts as originally hypothesized (Schwarz et al., 2006; Mendel, 2011).

To prevent sulfite toxicity within the plant, the plant produces the molybdoenzyme, sulfite oxidase. The primary function of Mo in sulfite oxidase is to act as the catalyst in the oxidation-reduction reaction by oxidizing sulfite to form sulfate and hydrogen peroxide (Schwarz et al., 2006; Mendel, 2011; Broadley et al., 2012). Since sulfite reductase is encased within the peroxisomes, the peroxisomes breakdown hydrogen peroxides and produces other free radicals before the byproducts can cause physical damage within the plant cell (Schwarz et al., 2006).

There are two main sources of sulfite accumulation in plants. First is through the breakdown of sulfur dioxide. Sulfur dioxide is a noxious gas produced globally as a byproduct of fossil fuel burning. It is also produced by natural processes, such as microbial respiration and geological activity (Schwarz et al., 2006; Brychkova et al., 2007; Broadley et al., 2012). Sulfur dioxide enters the plant through the stomata openings in the leaf. Consequently, as the number of open stomata increases, so does the concentration of sulfur dioxide entering the plant (Brychkova et al., 2007). Sulfite, another oxide produced in fermentation, can also be released from degradation of sulfur-based amino acids in higher level plants (Schwarz et al., 2006; Brychkova et al., 2007; Broadley et al., 2012). Although higher plants utilize small concentrations of sulfur dioxide for growth and development, elevated can cause internal cell damage.

Visual effects seen in the field from sulfur dioxide toxicity are chlorosis due to chlorophyll damage, which can lead to lower crop yields and plant death (Brychkova et al., 2007). Irrigated crops in fields with slow water penetration, heavy waterlogged soil, or peat-based mediums are more susceptible to sulfite toxicity (Hara, 2016). The saturated soils hold less oxygen and roots fast deplete this oxygen through respiration creating anaerobic conditions. Anaerobic bacteria present can reduce sulfate to plant-toxic hydrogen sulfite gas.

Aldehyde oxidase (XO oxidase family)

Higher level plants biosynthesize phytohormones to help regulate plant growth and development based upon the current environment. Among the five classes of phytohormones, two, abscisic acid (ABA) and indole-3-acetic acid (IAA), require the molybdoenzyme aldehyde oxidase (Engels, et al., 2012). Within the cytosol of the plant cell,

the Mo component of aldehyde oxidase acts as the catalyst in the redox reaction R-CHO + $H_2O \rightarrow$ R-COOH + $2H^+ + 2e^-$ (Mendel et al., 1999; Kaiser et al., 2005; Mendel, 2011).

In relation to ABA, the aldehyde oxidase redox reaction takes place during the final phase of biosynthesis. Mo oxidizes the organic compound abscisic aldehyde forming ABA (Schwarz et al., 2006; Yan et al., 2016). One of the many functions of ABA is to alert the other plant organelles of the environmental stress that will reduce growth and development. This allows the plant to respond appropriately and conserve internal resources to minimize tissue damage. For example, under drought conditions when the soil moisture is near plant wilting point, the roots will produce more ABA and transport ABA through the xylem to the leaves (Engels, et al., 2012). Once the leaves receive the ABA signal the stomata close to reduce water loss. Without the molybdoenzyme aldehyde oxidase, plants would wilt and yields possibly decreased.

Variable ABA concentrations also influence other plant responses to environmental stimulus. Plants with low ABA are often more sensitive to temperature changes, anaerobic conditions, saline soils, as well as produce less viable offspring (Mendel et al., 1999; Broadley et al., 2012). Research has shown that ABA plays an important role during seed maturation and seed dormancy. ABA is one of two phytohormones that choreograph protein and lipid storage within seeds and allows seed to remain in a dormant state until conditions are adequate for germination (Yan et al., 2016). Low Mo concentrations during this phase will prevent the future offspring from containing all the nutrients required for seed germination.

The second phytohormone, IAA, is a hormone growth regulator used to control most biological processes within the plant (Adoli et al., 2013). Since IAA is in the auxin family,

research suggests that aldehyde oxidase plays a more crucial role in IAA formation during the early development phases of higher level plant life cycles (Mendel, 2011). This means that IAA regulates the development of the plants vascular system, promotes cellular elongation, root growth, and aids in increasing plant height by increasing apical dominance, thus reducing lateral growth (Mendel et al.,1999; Adoli et al., 2013). Increased concentration of IAA also promotes increased plant growth by enlarging the plant leaves and aids in the transport of carbohydrates from the leaf to organelles (Abdoli et al., 2013). Therefore, a decline in aldehyde oxidase can lead to a lower leaf area index (LAI) as well as a reduction in plant sugar formation; i.e. lower brix values.

Xanthine dehydrogenase

The exact function of the molybdoenzyme, xanthine dehydrogenase, in higher level plants is still unclear. Most of the xanthine dehydrogenase research over the last few decades has focused efforts in the kingdom Animalia; specifically, within bovine milk (Mendel et al., 1999). The understanding of xanthine dehydrogenase in bovine milk has allowed scientists to confirm as well as disprove similar observations in higher level plants. What scientists have learned is that xanthine dehydrogenase is localized in both the cytosol and peroxisomes. Xanthine dehydrogenase has three plant functions: (1) aid in the formation of NADH (2) break down purines, the building blocks of DNA and RNA, to form uric acid (3) produces superoxides (Mendel, 2011). Since xanthine dehydrogenase controls the production of NADH and superoxides, some scientists hypothesize that xanthine dehydrogenase affects the ratio of $NADH/NAD^+$ used in the redox reaction for cellular electron transfer (Mendel, 2011). The exact details are still unclear.

What scientists are focusing on is research between the relationship of xanthine dehydrogenase with reactive oxygen species (ROS). Past research has revealed elevated concentrations of xanthine dehydrogenase and ROS in plants infected with pathogens as well as in threating abiotic growing conditions, specifically drought. It is possible that xanthine dehydrogenase is used to signal the plant in times of environmental stress; however, it is now postulated that xanthine dehydrogenase is present to begin disassembling purines in the senescence process (Mendel et al., 1999; Schwarz et al., 2006; Mendel, 2011). Hopefully within the next decade there will be a scientific breakthrough with both xanthine dehydrogenase and ROS.

Effect of molybdenum in plants

There are multiple publications outlining the growth and developmental effects from Mo on a variety of crops such as legumes and grain. However, there is limited published literature outlining the effects of Mo on muskmelon growth and development.

Legumes and Molybdenum

Prior research on Mo effects on germination rates, are focused on the physical outcomes between the interaction of Mo in different legume cultivars. Below are main findings from three independent studies using variable Mo concentrations with legumes in different growing environments.

In the first case study, a team of scientists at the University of Burdwan, India, placed Bengal gram or chickpea (*Cicer arietinum* L.) seeds on germination paper dampened with sodium molybdate solution (varying from 0-10 ppm Mo concentration) and allowed the seeds to germinate under optimal conditions (Datta et al., 2011). After ten days of incubation, a series of measurements were taken: root length, shoot length, wet weight, and dry weight. Datta and his team of scientists reached several conclusions, but three of most interest are:

(1) Applying a 3-4.5 mg/L Mo solution to *C. arietinum* L. seeds optimized the biological processes during germination; (2) Root and shoot lengths as well as nitrate reductase activity are maximized when seeds are germinated in a 4.5 mg/L Mo solution; and (3) Mo concentrations over 7.5 mg/L will reduce seedling root length by less than 20%; however, 7.5 mg/L Mo concentration will maximize chlorophyll a and b, and carbohydrate concentrations within the seedlings (Datta et al., 2011).

In the second case study, two scientists from University of Lucknow, India, studied the duration period for Mo toxicity/deficiency symptoms to appear in *C. arietinum* L., from planting to pod formation (Gopal and Shukla, 2016). Seeds were sown in containers holding five liters of clean sand and treated with sodium molybdate; each allowed to grow under optimal conditions inside a glasshouse. Since the duration of the study would exceed and exhaust the food storage provided by the cotyledons, plants were fertilized with a nutrient solution containing all required elements; except for Mo. After 100-days, Gopal and Shukla reached the following conclusions: (1) Visual symptoms of Mo toxicity first appeared at 35 and deficiencies at 42 days after planting; (2) *C. arietinum* L. with Mo tissue concentrations of 2.7-6.7 mg/kg produced the most pods per plant, seeds per plant, and highest dry matter yields; (3) germination and seedling growth was optimal at 2.7 mg/kg Mo; and (4) as long as the plant's internal Mo concentration was above 1.2 mg/kg, deficiency symptoms did not occur in chickpeas (Gopal et al., 2016).

In the third study, a team of scientists from both University of Sao Paulo and Sao Paulo State University, Brazil, monitored common bean plants, *Phaseolus vulgaris* L., cultivar Pérola, growing in 5x5 meter farm plots scatted throughout Mato Grosso do Sul State (Costa et al., 2014). Before planting, an N-P-K+Zn fertilizer was harrowed into the seed

beds. Additional 40 kg N ha^{-1} were side dressed 25 days after planting to ensure an adequate supply of nitrogen to finish the season. Three weeks after planting, *P. vulgaris* L. received a foliar application of sodium molybdate at a rate of 75 g ha^{-1}. After two years of trials in multiple plots throughout Mato Grosso do Sul State, scientists concluded that (1) most soils in the region contained adequate concentrations of Mo and applying extra Mo did not increase yield, (2) depending upon the location, foliar application of Mo to *P. vulgaris* L. increased seed size and yield, and (3) a low dosage of foliar sodium molybdate does not cause Mo toxicity in *P. vulgaris* L. (Costa et al., 2014).

These legume findings are extremely important because they provide a starting point for monitoring Mo effects in muskmelon seedlings. The collaboration of scientific legume research has demonstrated that seedlings uptake and utilize Mo from their growing environment, regardless of seed size and the initial Mo concentration within the cotyledons. This information suggests insights into ranges of Mo concentrations for possible treatments, the duration period for monitoring growth, and the parameters used to quantify effects of Mo on muskmelon seedlings.

Barley and Wheat with Molybdenum

Some agriculture regions grow grain crops, such as barley (*Hordeum vulgare* L.) and wheat (*Triticun aestivum* L.), in poorly drained soils that receive an influx of rain water or are flood irrigated regularly. In saturated soils, anaerobic conditions occur causing an increase in sulfide ions and a decrease in seed germination. In Japan, scientist Y. Hara coated both wheat and barley seeds with Mo trioxide at a rate of 0.05-0.5 mol-Mo/kg by weight (Hara, 2016). Seed of both cultivars were in the soil for two weeks under either flood or non-flooded conditions. The results were astonishing. By seed coating wheat and barley with Mo,

germination rose significantly. The influx of seed Mo allowed for an increase in sulfite oxidase production, thus reducing the sulfite toxicity for about four days. Hara concluded that Mo applications of 0.05-0.5 mol-Mo/kg improved germination in saturated soils from less than 5% to 89% in wheat, and up to 96% in barley. Under non-flooded conditions, the application of Mo did not significantly improve wheat or barely germination; and depending upon the Mo seed coat concentration germination was reduced (Hara 2016).

Since Mo increases germination in water logged soils, it is theoretically possible for Mo to increase germination in muskmelons under similar environmental conditions. This germination improvement is especially important for muskmelon seeds that are submerged under water for several days after flood irrigation, drip irrigated for 72 consecutive hours, or mass produced in greenhouses that use water saturated plugs and watered daily. The finding of Hara in both wheat and barley offer the possibility that seedling application of Mo to muskmelons may prevent sulfite-toxicity symptoms and stunted growth in over-irrigated muskmelons.

Muskmelon and Mo

The plant pathologist, W.D. Gubler, lead a two-year study in both San Joaquin Valley and Sacramento Valley, both in California, using multiple cultivars of muskmelons to determine the cause for yellowing and collapse of muskmelon vines. Gubler (1982) concluded that symptoms from Mo deficiencies can occur at any growth stage when the tissue levels drop below 0.5 mg/kg. These Mo deficiencies can occur from early runner through mature fruit. Although the symptoms are the same, the repercussions after recovery varied. Minor symptoms started with a thin chlorotic leaf margin on the mature crown leaves followed by interveinal yellowing. As Mo deficiencies intensified, crown leaves deteriorated

and senesced while symptoms advanced along the runners. Depending upon the growth stage and severity of the damage caused by the Mo deficiency, the yield response varied. At early runner or first fruit, few or no fruits are expected to set. Molybdenum deficiency occurring after fruiting or during mature growth stage reduced the average fruit size and decreased brix content. Plant tissue analysis was used to confirm Mo deficiency as the cause of plant disease. Tissue analysis from the third to fifth leaves from the growing node were analyzed for primary, secondary, and micronutrients content. In all cases, Mo was the limiting factor. To confirm this deficiency, Mo was applied to leaves at an application rate of 2.3 liter of a 23.0 g/kg sodium molybdate solution per hectare and 1.2 liters of a 40.0 g/kg sodium molybdate solution per hectare. Within 48 to 72 hours after foliar application the muskmelon crop recovered. In fact, vines previously diagnosed as Mo deficient at early runner/first fruit, begun fruit production just 24 hours after the foliar sodium molybdate application (Gubler, 1982).

CHAPTER 2

EFFECT OF MOLYBDENUM FERTILIZATION ON MUSKMELON SEEDLING GROWTH

Introduction

Muskmelon production in the Southwest

Southern Arizona soils are classified as skeletal soils and are typically sandy loam with a soil pH above 8.2. Although plant-available Mo increases with increasing soil pH, Mo is scarce in southwest soils. Molybdenum has been mined away as a result of generations of farmers implementing continuous year-round farming under irrigation. These soils have light texture, received minimal manure applications, and can hold approximately 1.2 mg/kg plant-available Mo by DTPA extraction. The remaining Mo in solution will leach through the soil profile to the wetting layer. Thus, crops lack adequate Mo to sustain metabolic activity.

Phosphorous helps maximize Mo uptake efficiency in plants (Datta et al., 2011), while sulfur decreases Mo uptake efficiency (International Plant Nutrition Institute, 2017). Phosphorus availability in Arizona is low due to high pH and calcareous soils. Moreover, the use of gypsum and sulfuric acid application for salt management increases available sulfur thus decreasing Mo availability for crop growth.

Molybdenum deficiency and requirements for muskmelon crops

Muskmelons (*Cucumis melo* L.) appear to have a higher Mo demand than other crops produced in Arizona (personal experience). Molybdenum deficiencies are difficult to diagnose because they often mimic nitrogen deficiencies or sodium toxicities (Gubler, 1982). Consequently, Mo-deficient fields are left untreated or treated incorrectly with commercial

products such as ammonium sulfate or UN32, urea nitrate-based fertilizer at 32% total nitrogen. Muskmelons cannot convert the absorbed nitrate-nitrogen into organic forms of nitrogen without Mo. When Mo deficiencies are present0, muskmelons continue to uptake and store the unusable nitrate-nitrogen in the leaf masking crop nitrogen deficiencies as well as causing plant tissue burn (International Plant Nutrition Institute, 2017). The only method to correctly diagnose Mo deficiency is through leaf tissue analysis at an agriculture laboratory.

Molybdenum deficiencies during pollination and seed development negatively affect the next generation seedlings in many crops (Costa et al., 2017). The effects are more severe in plants with larger seeds. The larger the seed, the greater the demand for Mo (Weir, 1984). Seeds from Mo deficient plants have poor viability and vigor during seed germination. One possible explanation is that molybdoenzymes are needed at multiple steps during the biological processes as seedling begin to initially grow and develop. Molybdoenzymes are needed to catalyze multiple pathways for nitrogen utilization, protein formation, and hormone production during germination (Gopal et al., 2017).

Low Mo concentration in the leaves is an indicator of Mo deficiency. Deficient plants have slow metabolic activity and reduced plant growth and development. Molybdenum deficiency soon after germination may lengthen the growth and development period and reduce plant vigor. The application of Mo fertilization could possibly shorten the muskmelon growth and development period, and possibly widen the planting window for Arizona muskmelon producers.

Muskmelons in Arizona, are planted mechanically by either direct-seeding or by seedling transplant. Producers irrigate direct-seeded muskmelons using subsurface drip or

grow their own transplants from seed in greenhouses on site. Shortening the amount of time required for transplant's to reach first or second true leaf will reduce labor costs, decrease water usage, and increase the number of muskmelon transplants produced overtime.

Planting is scheduled around weather events. Spring showers in Arizona are from February to March and monsoon storms are from July to August, while ambient temperatures also are favorable. However, sand storms and summer monsoon may damage entire fields requiring replanting. Faster germination rates and a shorter growth period can minimize hazardous weather effects.

Most research studies evaluate the role of Mo in acid, organic rich soils with ample soil moisture. There is little information, however, describing how Mo behaves in arid climates, like the Southwestern Sonora Desert. Therefore, the objectives of this study are:

Objectives

1. To determine the optimal Mo concentration in a foliar-application for reducing the initial seedling growth and development period in muskmelons.
2. To determine the optimal Mo concentration in a foliar-application for improving seedling vigor under greenhouse conditions.
3. To evaluate the net effect of Mo uptake in relation to total nitrogen, nitrate-nitrogen, carbon, and sulfur content within the seedlings.
4. To evaluate if Mo enhances or reduces nutrient uptake of the other essential nutrients.

METHODS AND MATERIALS

Preliminary Experiments

There is little information in the literature regarding Mo phytotoxicity in sandy soils. Consequently, a series of preliminary experiments were design to address possible phytotoxicity issues in muskmelon seed planted in the greenhouse experiment.

Effect of Mo concentration on seed germination

Muskmelon seeds were exposed to solutions with 10 different Mo concentrations to determine when Mo concentration in a solution becomes phytotoxic to seedlings. Ten muskmelon seeds were placed in between two paper towels moistened with one of 10 solutions with 0, 2, 4, 6, 8, 10, 12, 15, or 20-mg/L Mo concentration. The moistened towels containing seeds were placed inside an open plastic bag and placed inside an incubator without light at 25°C. After ten days, muskmelon seeds were removed from the incubator and visually examined to determine seed germination and length differences between Mo concentrations.

Effect of Mo concentration on seed germination in soil

To evaluate the effect of Mo fertilization on muskmelon seed germination in the field, a simple seed germination test was conducted using soil. Mineral soil from a local muskmelon farm in Harquahala, Arizona, was analyzed and amended to correct any nutrient deficiencies, toxicities, and nutrient ratio imbalances. The soil Mo concentration was also analyzed using DTPA (Diethylenetriaminepentaacetic acid) extract to confirm that no available Mo was present; <0.01 mg/kg Mo. Variable rates of Mo trioxide powder were incorporated into the soil, thus creating ten treatments (0, 2, 4, 6, 8, 10, 12, 15, or 20 mg/kg

available Mo). Five muskmelon seeds were planted into each container and irrigated with DI water as needed for two weeks. This two-week period allowed muskmelon seeds adequate time to germinate and display any phytotoxicity symptoms due to Mo exposure.

Determine movement of Mo in alkaline soils

To determine Mo movement and loss through the alkaline skeletal soil profile, soil from a local muskmelon farm in Harquahala, Arizona, was used. The soil was packed into two 10-cm long tubes and placed onto a perforated surface with a leachate collection cup below. Each tube received 25ml of a 30-mg/L water-soluble Mo solution. Two different sources of Mo were used in the solutions. Either sodium molybdate fertilizer or Mo trioxide were dissolved and brought to volume with DI water. Mo solution was allowed to percolate through the soil for three days. The leachate and the soil from each tube were analyzed by DTPA extraction using an ICP-OES for available Mo. The soil column was subdivided into 2.5-cm segments (0-2.5, 2.5-5, 5-7.5, and 7.5-10 cm) for analysis.

Initial Mo content in muskmelon seeds

To determine the nutrient content of a muskmelon seed, specifically molybdenum and sodium, fifty seeds per cultivar from the identical seed lot as used in the germination studies were dissected, removing the testa from the embryo. Per cultivar all the embryos were composited together and digested in aqua regia (3-parts hydrochloric 37% reagent grade and 1-part nitric acid reagent 70%) on a hot plate for four hours, cooled, brought to volume with DI water, and then analyzed for Mo and sodium by ICP-OES (inductively coupled plasma optical emission spectrometry).

Irrigation nutrient application

To assess uptake and seed germination when Mo is applied through irrigation, thirteen muskmelon seeds were planted in clean sand and irrigated daily with DI water containing Mo at a rate of 0, 2, 4, 6, 8, or 10-mg/L. Ten days after germination, seedlings and sand from each treatment were analyzed for molybdenum content. The seedlings were cut at the soil surface, dried, ground, digested in aqua regia, and analyzed by ICP-OES. The sand was extracted using DTPA and analyzed by ICP-OES.

Foliar nutrient application

The last preliminary trial compared the physical differences between plant growth and development using tap water verse reverse osmosis water. Nine muskmelon seeds were planted in clean sand and fertilized with an NPK solution once a week. Two days after the NPK fertilization, foliar Mo was applied at a rate of 0, 2, 6, or 10-mg/L. Only visual observations were made as muskmelon seedling grew for a period of three weeks.

Greenhouse Main Experiment

Based on preliminary research, a greenhouse experiment was conducted using two different cultivars of muskmelon seeds, Olympic Gold and Caravelle.

The greenhouse is located in the Sonoran desert at Inter Ag Services (IAS Laboratories) research greenhouse complex, Phoenix, Arizona. Atmospheric temperature of the greenhouse is regulated between 85-90^{o}F and cooled with a roof unit swamp cooler and blower located over the western side of the greenhouse. The eastern half of the south wall, east wall, and eastern half of the roof of the greenhouse are constructed of translucent plexiglass to allow for natural light and dark cycles. There is no internal electrical lighting system on site. The research was conducted during June through September and the outside

temperatures sometimes exceeded 115°F. A sunscreen shade cloth prevented internal greenhouse temperatures from reaching above 90°F.

The Mo research table had a chicken wire base to prevent excess heating of the soil substrate and to allow for proper soil drainage without the leachate causing interference with the other planting containers. The Mo research table was located along the north wall of the greenhouse under full sun. Four replications of twenty-four 4-liter planting containers were place on the table at one time. The replications were staggered over time separated by two-week intervals. The planting container layout was four containers across the research table by three containers deep. Each container was filled with approximately 3400 g of clean inorganic sand (Figure 1). Thirteen seeds of the same cultivar were planted in each planting container (Figure 1). Container-placement followed a randomized complete block design (RCBD) within the greenhouse (Figure 2).

Throughout the research, all 4-liter planning containers were irrigated with approximately 100 ml of tap water from Phoenix, Arizona. Before seedling emergence, all 4-liter containers received 100 ml tap water daily. After seedling emergence, the weekly irrigation schedule was as follows: Days 1-3 plants were irrigated with 100 ml tap water, Day 4 plants were not irrigated, Day 5 plants were irrigated with 100 ml of a 10 mg/L NPK fertilizer solution, Day 6 plants received 100 ml tap water, Day 7 received a foliar application of Mo. Tap water does not contain molybdenum but should supply some of the necessary nutrients, such as calcium and magnesium, to prevent root and seed rot as well as premature chlorosis that occurs with DI and reverse osmosis water. The foliar Mo solutions were administered weekly with a spray bottle, two days after the NPK fertilizer application. Each

Figure 1. Container configuration and seed placement. Courtesy of Sheri McLane (2018).

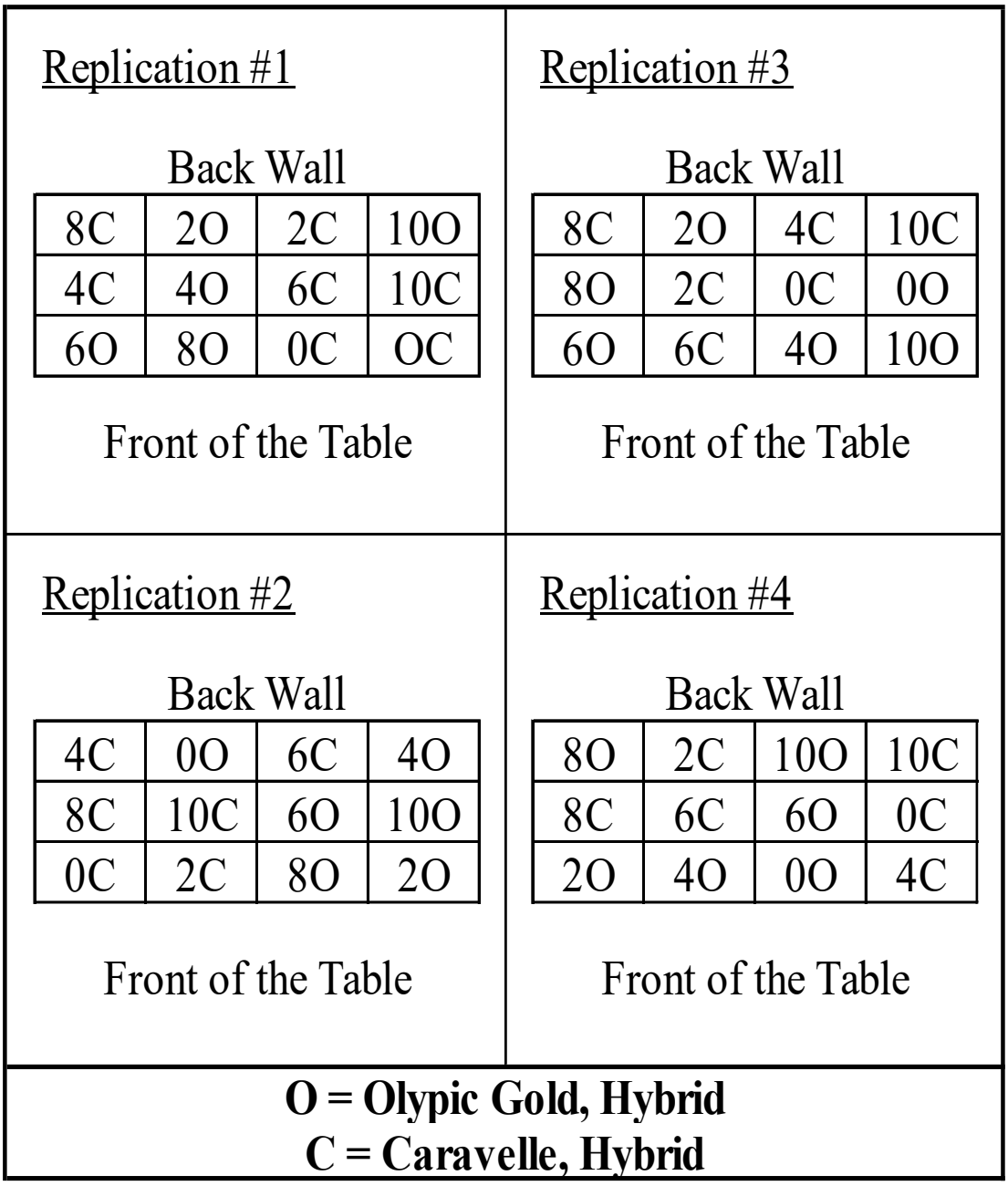

Figure 2. RCBD map for four muskmelon applications. Courtesy of Sheri McLane (2018).

4-liter container was isolated from the other 4-liter containers during the Mo application to prevent cross-contamination and then placed back into position on the greenhouse table top. Each container was misted ten times with the prescribed Mo treatment from a sterile spray bottle (Figure 3).

Sand Preparation

Prior to use in this study, the sand went through a two-step cleaning process. The sand was kilned at 500^{o}C for four hours to remove any organic matter or weed seed that may have been present. After kilned, the sand was washed thoroughly with DI water to flush any excess salts or nitrates. A subsample of clean sand was analyzed by IAS laboratories to insure no measurable plant nutrients remained. The soil analysis included pH, soluble salts, extractable calcium, sodium, potassium, magnesium, nitrate-N, phosphate-P, iron, manganese, zinc, copper, sulfur, boron, Mo, and free lime.

The sand pH was adjusted between 8.0-8.6 using 6 ml of reagent grade ACS sulfuric acid per 4-liter container and 400 ml tap water to mimic Arizona's alkaline pH soils.

Muskmelon Seeds

The two muskmelon cultivars, Olympic Gold and Caravelle, are commonly grown in southern Arizona. By using two hybrids instead of one, any differences between Mo uptake related to genetic variability will be revealed, thus eliminating a fixed effect. Seeds for this research were donated by Del Monte Fresh Produce and were stored at IAS Laboratories in the laboratory's seed and reagent refrigerator in the original packaging. Olympic Gold packaging was a sealed foil bad and Caravelle was foil lined canister.

Molybdenum Treatments

The source of Mo was reagent-grade Mo trioxide. Mo stock solution was made by

Figure 3. Muskmelon seedlings after first foliar molybdenum treatment. Courtesy of Sheri McLane (2018).

dissolving 5.000 grams of Mo trioxide into 2000 ml of DI water. The flask was covered with parafilm, and the solution was stirred on a hot plate for four hours at low heat to dissolve as much Mo as possible. Since Mo trioxide is not 100% water soluble, the stock was stored overnight at room temperature to allow settling of non-dissolvable particles. The next day, the Mo stock solution was analyzed for water-soluble Mo content using an ICP-OES.

The Mo concentrations in the solutions used for each treatment were: 0-mg/L (control), 2-mg/L , 4-mg/L , 6-mg/L , 8-mg/L , and 10-mg/L . Each Mo solution was made from the stock solution and brought to volume using DI water; then verified using an ICP-OES. The control consisted of DI water. The following formula was used to calculate each Mo solution:

(conc. stock sol.) × (vol. stock sol.) = (conc. Mo treatment) × (vol. Mo treatment)

Each of the six Mo treatments were replicated four times per cultivar using thirteen muskmelon seeds per 4-liter container.

Plant Growth Monitoring

The number of true leaves produced by each muskmelon seedling were counted and recorded. Only leaves approximately 1 cm in diameter were counted as a true leaf.

Root and Foliage Measurements

After four weeks from the initial irrigation, the sand was washed away exposing the root growth. Root and shoot lengths were measured and recorded to the 0.0025 cm with a Cent Tech P5658 dial caliper. Root length measurements started from the closest root to the soil surface attached to the stem and proceed to the longest growing point, the root cap. Both primary and secondary roots were analyzed as one unit. Shoot length measurements were taken of the stem beginning at the soil line and ending at the base of the newest leaf.

Seedling Fresh and Dry Weights

After the completion of length measurements, both seedling fresh and dry weights were measured using a HR-A series analytical balance with 0.0001g precision. Before seedling fresh weight measurements were taken, all cotyledon tissue was removed and seedlings were blotted dry to remove any external moisture. All seedlings were placed into a pre-weighed envelope for measurement. The seedlings were dried inside the envelopes at 80ºC until constant weight. Dry weights were calculated as follows, expressing the results in mg/seedling:

(Total dry weight of the seedlings)/(number of seedlings dried)

Nutrient Analysis

Dried seedlings were ground to a homogenous powder passing sieve #40 using a mini Wiley mill grinder. The ground seedlings were split into three subsamples. Each subsample was processed one of three ways: (1) Total nitrogen and total carbon values were analyzed by TruSpec CN analyzer by combustion at 950ºC using method AOAC 993.1, (2) Total sulfur and Mo were analyzed by ICP-OES using method EPA 3050B, and (3) Nitrate-nitrogen was extracted using a 2% acetic acid solution and then analyzed by spectrophotometer with a Skalar San^{++} System at 540 λ .

Statistical Analysis

The effects of variety, Mo concentration, and their interaction were determined by analysis of variance (Clewer et al., 2001) using Excel (Appendix A and Appendix B). Model effects and subsequent mean comparisons were evaluated at a fixed alpha = 0.05. Comparison of treatment means was based on the least significant difference (LSD)

calculated from the error variance. Nutrient graphic analyses were conducted using scatter plot to determine nutrient correlations with Mo by evaluting linear regressions.

RESULTS

Preliminary Experiments

Effect of Mo concentration on seed germination

When paper towels were moistened with DI water and no Mo, seeds did not germinate. Seed imbibition was evident in the control and resulted in seed coat from some seeds to open 1-2 mm without root elongation (Figure 4). The hypocotyl from seeds imbibed with the 4-mg/kg Mo solution grew an average of 5 to 6.5 cm and the radicle length was 7 to 10 cm (Figure 5). Muskmelon seeds germinated and did not have phytotoxicity symptoms, regardless of Mo concentration in the solution. However, the radicles of seedlings grown in the 20-mg/L Mo solution were half the length, 4.3 cm, than those from seedlings germinated with the 4-mg/L solution.

Effect of Mo concentration on seed germination in soil

The Mo concentration in the mineral soil did not affect germination. Every seed in all ten treatments, including the control, germinated within 8-12 days.

Determine movement of Mo in alkaline soils

Analytical results indicated that the Arizona alkaline soil can hold a maximum of 1.1-1.3 mg/kg plant-available Mo. The remaining Mo was deposited where the water flow stopped; in this study it was the leachate. Mo concentration at 0-2.5 cm was 1.2 mg/kg and at 7.5-10 cm was 1.3 with the leachate holding 23.7 mg/L Mo.

Initial molybdenum content in muskmelon seeds

Chemical analysis of the embryos revealed that the seed contained 28-56 ppm Mo and less than 0.01 g/kg sodium.

Figure 4. Muskmelon germination results with 0-mg/L molybdenum.

Courtesy of Sheri McLane (2018).

Figure 5. Muskmelon germination results with 4-mg/L molybdenum.

Courtesy of Sheri McLane (2018).

Irrigation nutrient application

Every seed germinated within 3-4 days. After 10 days, all plants, including the control, exhibited severe chlorosis. Chemical analysis of the inert sand and plant tissue revealed several things: (1) Clean sand can hold an average of 1.1 mg/kg Mo. (2) Plants irrigated daily with Mo uptake large amounts of Mo though the roots. Seedlings receiving 0-mg/L Mo contained 13.2 mg/kg Mo in the tissue and seedlings irrigated with 10-mg/L Mo contained 15,193 mg/kg Mo. (3) All seedlings became toxic with tissue sodium concentration at 19 g/kg and deficient for calcium with total calcium tissue concentrations at 17 g/kg.

Foliar nutrient application

Ten days after the first irrigation, the seedlings remained green using tap water. Then three weeks after first irrigation, subtle differences in first true leaf size were seen between the varied Mo treatments. Chlorosis was also beginning to occur, but not as severe when visually compared to seedlings receiving Mo through daily irrigation. Now, the main question is: How does Mo effect growth and development in muskmelon seedling? Using all the compiled preliminary research to reduce experimental error as well as prior research with legumes, a finalized protocol was developed to answer the question.

Greenhouse Main Experiment

Leaf Number

Muskmelon cultivar, Caravelle, showed a significant difference between the 0-mg/L, 2-mg/L, and the 4 mg-L Mo treatment with 6-mg/L, 8-mg/L, and 10-mg/L Mo treatments. The control and two lowest Mo treatment grew 2.0 leaves while the three highest Mo treatments developed 2.3 leaves. There was; however, no significant difference in leaf

number between variable molybdenum concentration with the muskmelon cultivar, Olympic Gold (See Table 1).

Root Growth

Significant differences occurred in root length in both muskmelon cultivars. Olympic Gold seedlings from control treatments were significantly shorter than seedlings treated with 2, 6, 8, and 10-mg/L Mo, but not different from 4 mg/L Mo. Only seedlings receiving a foliar application of 8 and 10-mg/L Mo had the longest roots (Table 1). In Caravelle, roots from seedlings treated with control and 2-mg/L Mo treatments were significantly shorter than those from 8-mg/L Mo treatment. For both cultivars, 8-mg/L Mo treatment grew the longest roots (Table 1).

Shoot Growth

There were no significant differences in shoot length among seedlings from all Mo treatments in both muskmelon cultivars (Table 1).

Seedling Wet and Dry Weight

Only wet and dry weights of Caravelle seedlings between the 2-mg/L and 8-mg/L Mo treatments were significantly different (Table 1). The wet and dry weights of seedlings from Olympic Gold cultivar were not significantly different regardless of Mo treatment (Table 1).

Nutrient Analysis

Molybdenum

The Mo concentration was significantly different in Olympic Gold cultivar seedlings. Mo concentrations in control seedlings were lower than in seedlings treated with Mo solutions (Table 2).

Table 1. Physical Parameters. Physical testing parameter results for both muskmelon cultivars, Olympic Gold and Caravelle. Means followed by same letter within the cultivar and testing parameter are not significantly different (P=0.05) as determined by LSD.

Physical Testing Parameters					
Molybdenum Treatment Concentration (mg/L)	Number True leaves	Root Length (cm)	Shoot Length (cm)	Wet Weight (g)	Dry Weight (g)
Cultivar: Olympic Gold					
0	2.0 a	13.865 c	14.066 a	1.7229 a	0.1448 a
2	2.0 a	15.023 ab	14.306 a	1.8030 a	0.1481 a
4	2.0 a	14.237 b	13.088 a	1.6261 a	0.1256 a
6	2.0 a	14.785 ab	12.805 a	1.6487 a	0.1274 a
8	2.1 a	15.399 a	13.633 a	1.8839 a	0.1374 a
10	2.1 a	15.173 a	14.517 a	1.8413 a	0.1434 a
Cultivar: Caravelle					
0	2.1 ab	13.686 b	12.188 a	1.7146 ab	0.1398 ab
2	2.0 b	13.345 b	11.717 a	1.6048 b	0.1308 b
4	2.1 ab	14.715 ab	12.177 a	1.7568 ab	0.1456 ab
6	2.3 a	13.764 ab	12.693 a	1.8001 ab	0.1469 ab
8	2.2 ab	15.652 a	13.025 a	1.9809 a	0.1614 a
10	2.3 a	14.407 ab	12.926 a	1.7767 ab	0.1456 ab

Table 2. Growth parameters. Nutrient concentration of the entire seedling (roots and shoots combined) for both muskmelon cultivars, Olympic Gold and Caravelle. Means followed by same letter within the cultivar and testing parameter are not significantly different (P=0.05) as determined by LSD.

Nutrient Concentration in Muskmelon Tissue						
Molybdenum Treatment Concentration (mg/L)	Molybenum (mg/kg)	Nitrate-Nitrogen (mg/kg)	Total Nitrogen (g/kg)	Total Carbon (g/kg)	Sodium (g/kg)	Calcium (g/kg)
Cultivar: Olympic Gold						
0	1.161 b	320.76 a	10.71 a	348.03 a	25.59 b	13.25 a
2	1.918 a	216.75 b	10.56 a	343.63 a	27.91 ab	12.60 a
4	2.137 a	337.05 a	11.16 a	339.71 a	33.10 a	12.45 a
6	2.078 a	282.81 ab	10.61 a	339.86 a	32.98 a	13.29 a
8	2.058 a	295.02 ab	10.55 a	345.02 a	30.10 ab	13.96 a
10	2.428 a	360.28 a	10.50 a	342.78 a	29.63 ab	14.06 a
Cultivar: Caravelle						
0	1.173 d	321.37 ab	10.91 a	338.95 b	31.18 a	14.18 a
2	2.072 bcd	283.53 b	10.90 a	349.32 a	27.28 a	14.33 a
4	1.909 cd	324.13 ab	11.67 a	348.90 ab	26.56 a	14.17 a
6	2.404 ab	320.09 ab	11.77 a	347.94 ab	28.23 a	14.31 a
8	2.582 a	335.37 ab	10.92 a	343.55 ab	28.87 a	14.01 a
10	2.296 abc	352.12 a	12.04 a	350.26 a	28.52 a	14.41 a

There were significant differences in the Caravelle cultivar tissue analysis among Mo treatments. Tissue concentrations of Mo were highest in the 8-mg/L Mo treatment. However, this value was not significantly different from treatments 6-mg/L and 10-mg/L (Table 2).

Nitrate-nitrogen and total nitrogen

Table 2 shows the concentration of nitrate-N and total N in seedling tissue for the different treatments and cultivars. While there were no significant differences in total N among treatments in both cultivars, there were some differences in Nitrate concentrations. There were significant differences in the amount of nitrate-nitrogen recovered from seedling tissue of both cultivars. Seedling tissue from 2-mg/L Mo treatment were lowest while 10-mg/L were highest (Table 2).

Total carbon

There was a significant difference of total carbon in the Caravelle seedling tissue between the control treatment (0-mg/L) and 2-mg/L and 10-mg/L Mo treatments. The 2-mg/L Mo treatment contained 349.32 g/kg Carbon and 10-mg/L Mo treatment contained 350.26 g/kg carbon (Table 2). There was; however, no significant difference in the total carbon concentrations in tissue from Olympic Gold (Table 2).

Calcium

There were no significant differences in calcium concentrations in seedling tissues for Mo treatments and their interactions (Table 2).

Sodium

The interaction of Mo concentration within Olympic Gold cultivar was significant for sodium concentration in muskmelon seeding tissue. Sodium concentration for the 0-mg/L

control was 25.59 g/kg with 4-mg/L Mo treatment at 33.10 g/kg and 6-mg/L Mo treatment at 32.98 g/mg. There was; however, no significant difference in the sodium concentrations found in Caravelle seedling tissue (Table 2).

Correlations between different nutrients

Figure 7, 8 and 9 show the relationship between Mo content in seedlings and zinc, boron, and sulfur, respectively. The correlation between these elements and Mo in the seedling tissue was high. The r^2 for the correlation of these nutrients and Mo were close to 0.9.

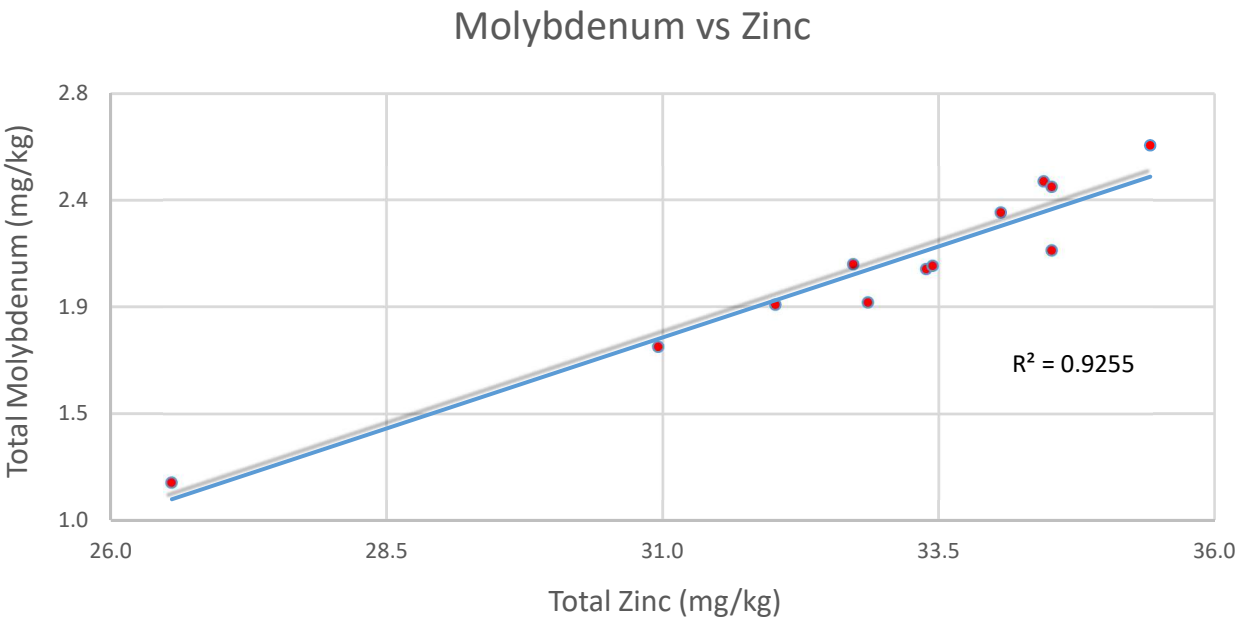

Figure 6. Linear regression of total molybdenum concentration predicted by total zinc concentration in muskmelon seedlings. Courtesy of Sheri McLane (2018).

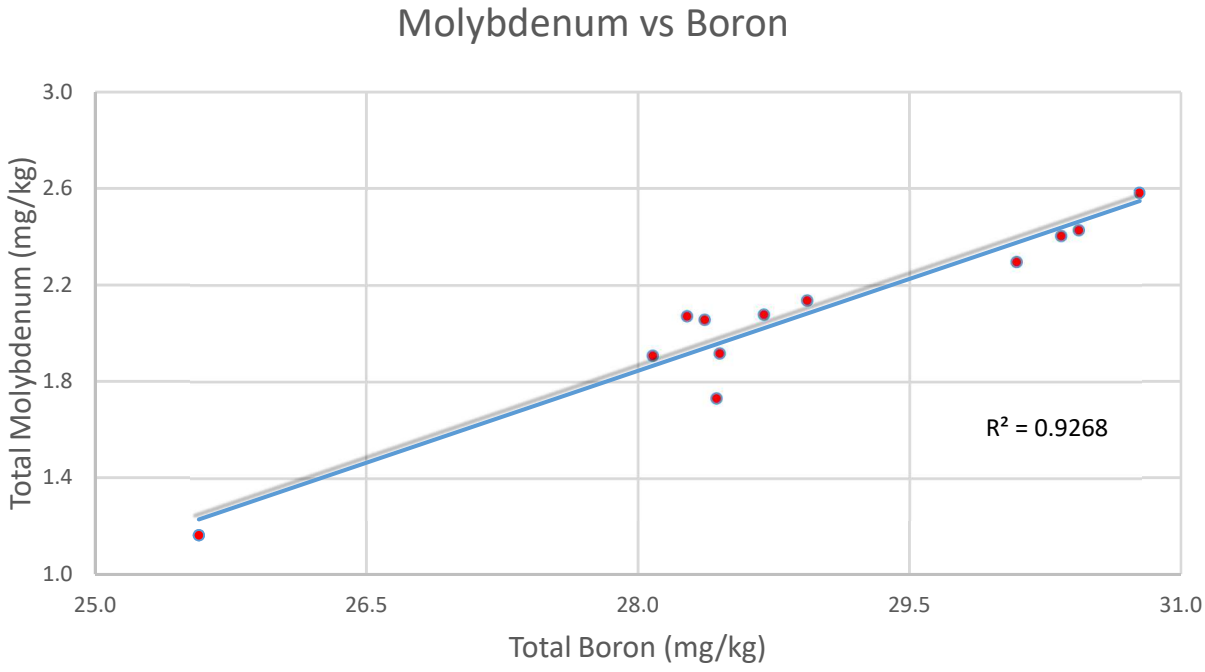

Figure 7. Linear regression of total molybdenum concentration predicted by total boron concentration in muskmelon seedlings. Courtesy of Sheri McLane (2018).

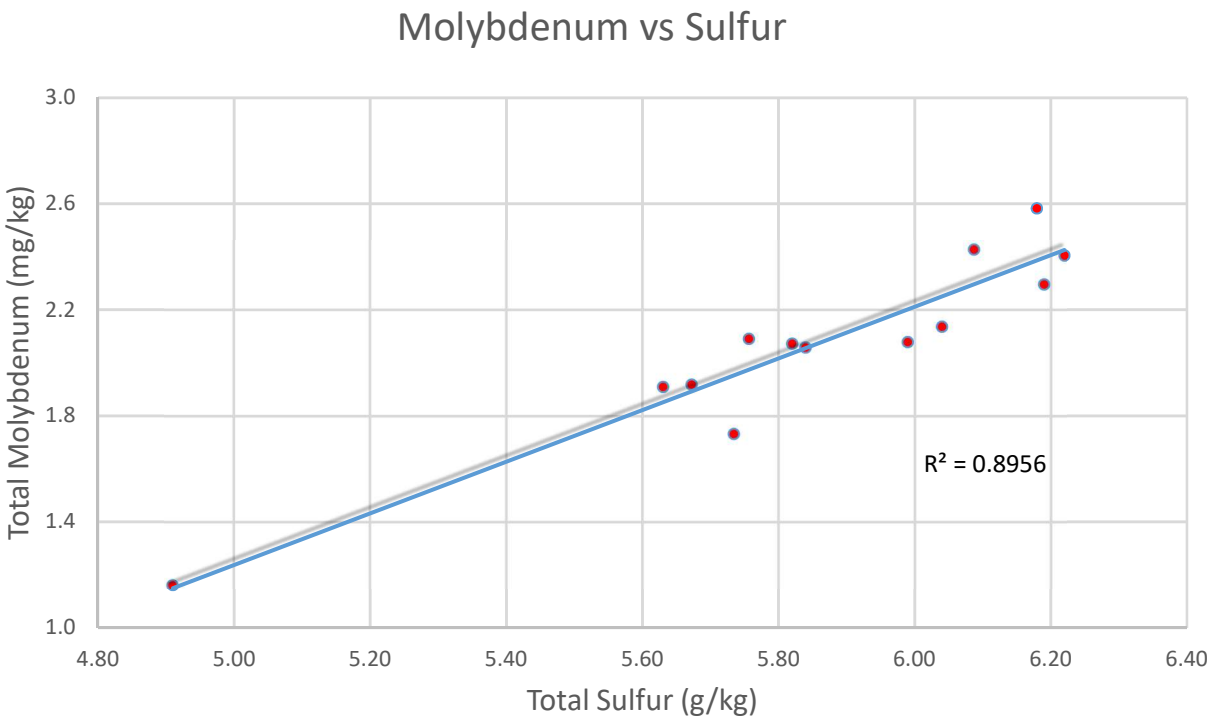

Figure 8. Linear regression of total molybdenum concentration predicted by total sulfur concentration in muskmelon seedlings. Courtesy of Sheri McLane (2018).

DISCUSSION

Preliminary experiment

The Mo concentration in the solutions from the preliminary experiment did not create phytotoxicity symptoms in muskmelon seedlings. Even though higher concentrations of Mo stunted seedlings growth, only using DI water negatively affected germination. When seed were planted either in sand or alkaline soil, both cultivars germinated regardless of Mo treatments.

The maximum limit of Mo adsorption was not reached in these preliminary experiments. The seedlings continually adsorbed inexhaustible amounts of Mo when Mo fertilization was provided daily through the irrigation water. When grown in sand, muskmelon seedlings in all Mo treatments were negatively affected by abiotic factors such as high sodium and low calcium.

Based on Mo studies in legumes (Datta et al., 2011; Costa et al., 2014 ; Gopal et al., 2016) and the preliminary results, the initial experiment (foliar nutrient application) was designed to study the effects of Mo on muskmelon seed germination. Since alkaline soils cannot hold more than approximately 1.2-mg/kg Mo (according to the preliminary results), seeds were planted in sand and irrigated daily with different Mo solutions. However, due to unexpected turn of events, ten days after germination, all seedlings from both cultivars suffered severe chlorosis. Soil and tissue analysis indicated that Mo was up taken by the roots of the seedlings and was not accumulating in the sand. Tissue analysis also revealed toxic levels of sodium and deficient levels of calcium; both abiotic facts that can cause

chlorosis. Chemical evaluation of both the seedlings and growing medium for each Mo treatment provided guidance and a new direction for evaluating Mo effects on muskmelons.

Seedlings in the control group contained ten to fifty times more Mo than mature leaves from farm fields at six weeks after planting. Seed and soil analysis determined that seed contained adequate Mo to begin the germination process, but that sodium from an undetermined source accumulated in the soil and seedlings. Both seed content and pre-plant soil analysis indicate low or non-detectable concentrations of sodium.

Greenhouse Main experiment

Muskmelon growth and development

Molybdenum foliar fertilization did not significantly improve muskmelon seedling growth and development, regardless of cultivar. Different abiotic factors could have influenced this lack of response. For example, low calcium, high sodium, or deficient nitrogen could have influenced muskmelon growth, providing the lack of Mo biological activity response (Laarayedh et al., 2009).

Low Calcium

The expected calcium concentration in leaf tissue at early runner growth-stage ranges between 30.0-50.0 g/kg (Bryson, 2014). Low calcium concentrations may reduce plant growth and development as well as interfere with root and shoot elongation (Gao et al., 2011; Hawkesford et al., 2012). Calcium is an important building block in cell wall formation. As calcium becomes less available, the plant will utilize other cations in leu of calcium, such as sodium. As calcium deficiency goes untreated, the cell membrane weakens, and plants become susceptible to cell degradation, losses of photosynthates and other nutrients occur, and disease resistance declines (Hawkesford et al., 2012). In our greenhouse experiment,

muskmelon seedlings could have exchanged the calcium cation for sodium, as indicated by the high sodium levels in the seedling tissue. Their sodium concentration was nearly 30.0 g/kg, which is more than ten times the typical sodium concentration (2.5 g/kg) observed in normal muskmelon tissue sampled from horticulture production fields or the 5.0-4.2 g/kg cited in the literature (Laarayedh et al., 2009; Turcios et al., 2016).

Another indication of possible replacement of calcium by sodium were the occasional circular brown spots in the newest true leaves. These spots appeared 24 days after planting and appeared on both sides of the leaf. Although not confirmed, the brown spot did appear to be biotic in nature; possibly a secondary fungal infection from low calcium (Hawkesford et al., 2012). The seedlings did not present any additional pathology, but within a couple of weeks the leaf and petiole senesced. The addition of calcium, many times, resolves these leaf symptoms (Gao et al., 2011; Hawkesford et al., 2012).

Calcium deficiencies also inhibit cellular elongation. In general, higher level plants low in calcium have smaller leaves, shorter stems and roots without root hairs (Hawkesford et al., 2012). In our experiments, the total calcium concentration in the muskmelon seedlings seemed to play a role in inhibition of cellular elongation. For example, Mo treatments of 8-mg/L and 10-mg/L for Olympic Gold and 8-mg/L treatment for Caravelle had the longest roots and stem lengths.

Muskmelon roots from harvested seedlings were shallow and only penetrated the top four-inch depth within the 4-liter containers. Roots were several mm thick, kinked, and with minimal root hairs. These symptoms could be linked to low calcium concentrations both, within the seedlings and in the sand (Lynch et al., 2012). The sand used in these experiments had an initial available calcium concentration of 2000 mg/kg and had medium free lime

content. Since the overall salinity of the sand was within normal levels with a value of 1.5 dS/m, there should have been adequate calcium available for plant growth. However, seedlings presented symptoms of calcium deficiency. Gypsum, a water-soluble source of calcium, could be used to amend low plant available calcium concentrations, at a rate of 2200 kg of gypsum per hectare before planting (Lynch et al., 2012). Future research should explore the effect of calcium amendment on seedling health.

Analysis of the irrigation water used in these experiments revealed water soluble calcium content was 55 mg/L. The optimum concentration in irrigation water is no less than 40 mg/L soluble calcium (Will et al., 2017). Lower calcium concentrations and high sodium can reduce water penetration and form soil crusting, reducing gas exchange between the atmosphere and soil (Bauder et al., 2011). If not corrected, anaerobic conditions in the soil can alter the growing environment for roots that can lead to root rot and a decreasing root mass (Lynch et al., 2012).

The initial pH of the sand used in these experiments was 11.8. Before using the sand, the pH was adjusted to 8.1 to simulate growing conditions in the Arizona. The sulfuric acid used to adjust sand pH could have dissolved the calcium, free lime, carbonates, and bicarbonates in the soil releasing trapped sodium. During this chemical reaction, the sulfur in the sulfuric acid could have reacted with the dissolved calcium and form gypsum. Irrigation water was applied to flush the excess sulfur and salts from the sand to prevent salt burn. Unfortunately, sand has a reduced buffering capacity and the available calcium could have been flushed along with the excess salts (Brady et al., 2008). The calcium that was present in the irrigation water was not enough to build up the calcium concentration in the soil over the duration of the Mo trial to improve muskmelon growth and development (Table 2). Future

research should evaluate the effect of adding 1.5g of gypsum applied per 3400g of sand after adjusting the soil pH.

High Sodium

The initial soil analysis also indicated a plant available sodium concentration of 190 mg/kg, which is well below the ideal higher limit of 300 mg/kg. Also, the irrigation water analysis revealed 150 mg/L sodium. Since sodium is a mobile element, irrigating with extra water should flush the excess out of the 4-liter containers. However, whole plant tissue analysis revealed something unexpected. All muskmelon seedling growing under all treatments of Mo, including the control, contained high levels of sodium. The seedlings accumulated 29.0$\pm$4.0 g/kg sodium, compared with 0.30 g/mg present in the soil.

When plants contain too much sodium, plant growth and development is restricted due to a reduction in photosynthesis. Sodium also blocks the uptake of other necessary cations, specifically calcium, magnesium, potassium, and phosphorus (George et al., 2012). These primary and secondary nutrients are required for cellular membrane formation. They are also essential to the cell's osmotic regulation, transport of solutes, and DNA formation (George et al., 2012). Visual symptoms of sodium toxicity are yellowing of plant tissue, reduced root growth, and smaller leaf size. These exact toxicity symptoms were observed in both cultivars of muskmelon seedlings from all Mo treatments, including the control, providing additional evidence that sodium toxicity could have been an issue for the muskmelons.

To detect the source of sodium in these experiments, all components were analyzed. Pre-planting analysis ruled out the sand and irrigation water as possible sources of sodium. Analysis of the irrigation water and seed confirmed that they did not contain sodium. We

hypothesized that the sand was the source of sodium after adjustment of pH with sulfuric acid. After flushing the excess salts, crusting formed on the soil surface. The crust was broken up and removed from the sand surface before seedling emergence. Future research should evaluate the effect of added gypsum or soluble calcium on sodium accumulation in the muskmelon seedlings. This calcium amendment could be applied either directly to the sand prior to planting or added in the irrigation water after planting.

Nitrate-Nitrogen and Total Nitrogen

Nitrogen deficiency could also have masked the symptoms of molybdenum deficiency in these experiments. Over the course of this experiment, seedlings received the equivalent of 5 kilograms of nitrogen per hectare. The total nitrogen applied was subdivided into four applications to prevent seedling burn and to provide uniform supply of nitrate-nitrogen during initial growth and development. However, when compared to normal field fertilizations, this application of nitrogen could be considered too low. Under field conditions in Arizona, an equivalent of 30 kilograms of nitrogen per hectare is applied either at pre-planting, or split-applied with each irrigation until the seedlings reached early runner stage. To achieve these levels of nitrogen fertility, 200-ml of a 30-mg/L nitrate solution should have been added to each pot. Low nitrogen fertility symptoms included stunted growth and development, reduced leaf size, and yellowing of the foliage (Hawkesford et al., 2012). All these symptoms were observed in both cultivars of muskmelons. Analytical testing of both nitrate-nitrogen and total nitrogen in seedlings from all molybdenum treatments indicated nitrogen deficiencies. Petiole analysis of early runners should range between 8,000-12,000 mg/kg (Bryson, 2014). This author also indicated that adequate levels of total nitrogen in the leaf tissue should be in the range of 45-55 g/kg (Bryson, 2014).

However, in this experiment, the analysis was conducted on the entire seedling. Nitrate-nitrogen levels were non-detectable and total nitrogen values were only a fourth of the anticipated level. One possible explanation is that all the inorganic nitrate-nitrogen taken up by the plant was metabolized into organic nitrogen via the molybdoenzyme, nitrate reductase (Mendel, 2011). Further research should explore how nitrogen fertilizer applications may affect the overall nitrate-nitrogen and total nitrogen concentrations in muskmelon seedlings. It is possible that increasing nitrogen application rates could cause significant differences in growth and development among treatments.

Correlations between nutrients

Plant uptake of Mo also enhances the uptake of two micronutrients and one secondary nutrient: zinc, boron, and sulfur, respectively. Specifically, as the concentration Mo increases in the overall plant, the concentration of these three elements also increases.

Sulfur

The analytical results in this experiment were performed on a composite sample of the entire plant. Future research should investigate the accumulation of these minerals in different parts of the plant. If the root tissue, stems, and leaves were analyzed separately, then we could determine whether sulfur and Mo accumulation occur predominantly in the roots or is distributed equally throughout the plant. Higher accumulation of sulfur and Mo in roots could be associated with root nutrient uptake (Mendel, 2011). Future research also should include Mo application in the irrigation water to elucidate its role in absorption through the roots.

Sulfur has many important roles in plant growth and development, including the production of chlorophyll and proteins. Mo has five bonding sites, three of which are

chemically bonded directly with sulfur molecules to form the Mo cofactor Moco. Mo has the biological function of forming a pterin (proteins) complex or Moco. Moco is then inserted into molybdoenzymes, such as nitrate reductase, to complete redox reactions necessary for biological activity (Mendel, 2011). This Mo-S relationship is found in all molybdoenzymes (Schwarz et al., 2006). It is highly possible that the positive correlation between Mo-S found in the muskmelon seedlings is associated with the formation of nitrate reductase. This S-containing enzyme is synthesized in preparation for the metabolic conversion of nitrate from recent fertilizer applications into usable forms of nitrogen. To verify this hypothesis, specific enzyme identification and concentration in the tissue are required as well as separate analysis of the root, stem, and leaf.

As these molybdoenzymes and other sulfur compounds break down within the plant, sulfur is released in the form of sulfite, which is toxic to plants. To prevent phytotoxicity, the molybdoenzyme, sulfite oxidase, oxidizes sulfite into sulfate (Schwarz et al., 2006; Mendel, 2011; Broadley et al., 2012).

In this experiment, the nitrate-nitrogen concentration in the muskmelon seedlings was very low compared with petiole nitrate-nitrogen concentrations of field production melons at early runner growth stage. It is possible that excess of the nitrate reductase oxidized Moco and released sulfur as well as free radicals (Mendel, 2011). During this degradation process sulfur would have been released as the molybdoenzyme breaks down, an influx of sulfite could have triggered the formation of the sulfur oxidase, another molybdoenzyme, to reduce sulfite toxicity within the seedlings (Schwarz et al., 2006; Brychkova et al., 2007; Broadley et al., 2012). Future research can validate this suspicion by using X-ray diffraction.

Zinc

Although zinc is required in the formation of many enzymes, it is also involved the production of IAA, a process that requires the molybdoenzyme aldehyde oxidase (Tsonev et al., 2012). IAA is a plant hormone which promotes cell and plant elongation (Abdoli et al., 2013). As the concentration of zinc increases in the seedling, the amount of IAA produced also increases (Broadley et al., 2012; Abdoli et al., 2013), thus promoting aldehyde oxidase production. As the hypocotyl arch emerges from the sand, cellular elongation of the stem begins as the muskmelon cotyledons reach for sunlight. IAA production in melons continues as cellular elongation promotes growth of roots, long runners, and large leaves to form its canopy before setting flowers (Kaveh et al., 2011).

Boron

Boron plays a significant role in plant growth and development, aiding in secondary nutrient uptake required for cellular strength as well as promoting tiller and seed head formation in grain. Boron can also block IAA effectiveness in plants. Prior research has shown that a decrease in IAA can lead to a reduction in root growth with increasing boron levels (Broadley et al., 2012). In the current Mo research, the muskmelon root formation was thick, kinked, with no visual root hairs; however, the reason for a positive correlation between Mo and boron in relation to IAA is unclear.

Since boron plays a significant role in meristematic activity as well as affects the metabolism of nitrogen with nitrate reductase activity, it is highly probable that the possible correlation between boron and Mo lies here (Cervilla et al., 2009). The low nitrate-nitrogen concentrations in the muskmelon tissue indicated the possibility that all inorganic nitrogen

taken up by the seedlings was converted to organic forms of nitrogen possibly following the pathway that utilizes the enzyme nitrate reductase.

CHAPTER 3

CONCLUSIONS

Foliar application of 8-ppm Mo solution to muskmelon seedlings increased root growth and development and increased uptake of sulfur, boron, and zinc. When producing muskmelons from seed, an increase in root length can help new seedlings penetrate the soil faster in search of nutrients and water. This improved root system is very important for seedling establishment and enhances seedling early growth. This improvement in crop establishment will help growers producing melons in arid climates, where soils often are calcareous and sodic in nature and crops are irrigated.

Future greenhouse research into the effects of Mo on muskmelon seedlings should include removal of free sodium after pH of the media is corrected. Also, gypsum should be broadcasted over the media before planting and added to the irrigation water. This gypsum application will provide calcium to both the substrate and seeds for optimal growth and development. Analyzing the tissue from root and shoot separately will also allow better understanding of the role of Mo absorption by the root.

Field trials using variable foliar molybdenum will evaluate seedling growth and development in a cultivation setting. Nutrient correlation between Mo with boron, zinc, and sulfur can be further evaluated with the possible saving to the farmer by reducing fertilizer inputs.

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APPENDIX A

ANOVA TABLES – OLYMPIC GOLD CV.

Analysis of variance for leaf number of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	0.059	5	0.012	0.9514	2.7729
Error	0.225	18	0.012		
Total	0.284	23			

Analysis of variance for root length of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	6.891	5	1.378	3.9868	2.7729
Error	6.222	18	0.346		
Total	13.113	23			

Analysis of variance for shoot length of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	9.802	5	1.960	0.5678	2.7729
Error	62.150	18	3.453		
Total	71.952	23			

Analysis of variance for wet weight of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	*SS*	*df*	*MS*	*obs. F*	*F (P=0.05)*
Treatment	0.221	5	0.044	1.0456	2.7729
Error	0.762	18	0.042		
Total	0.983	23			

Analysis of variance for dry weight of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	*SS*	*df*	*MS*	*obs. F*	*F (P=0.05)*
Treatment	0.002	5	0.0004	0.9396	2.7729
Error	0.007	18	0.0004		
Total	0.009	23			

Analysis of variance for molybdenum of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	*SS*	*df*	*MS*	*obs. F*	*F (P=0.05)*
Treatment	3.653	4	0.913	7.3895	2.6207
Error	2.348	19	0.124		
Total	6.001	23			

Analysis of variance for nitrate of Olympic Gold cultivar muskmelon seedlings treated with a with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	63308.341	5	12661.668	2.2828	2.6207
Error	133118.785	24	5546.616		
Total	196427.127	29			

Analysis of variance for total nitrogen of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	0.012	4	0.003	0.6238	2.6207
Error	0.091	19	0.005		
Total	0.102	23			

Analysis of variance for total carbon of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	2.515	5	0.503	0.6162	2.6207
Error	19.591	24	0.816		
Total	22.106	29			

Analysis of variance for calcium of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	0.089	4	0.022	1.1950	2.6207
Error	0.353	19	0.019		
Total	0.442	23			

Analysis of variance for sodium of Olympic Gold cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	1.696	4	0.424	1.9300	2.6207
Error	4.173	19	0.220		
Total	5.869	23			

APPENDIX B

ANOVA TABLES - CARAVELLE CV.

Analysis of variance for leaf number of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	*SS*	*df*	*MS*	*obs. F*	*F (P=0.05)*
Treatment	0.278	5	0.056	1.822	2.7729
Error	0.550	18	0.031		
Total	0.828	23			

Analysis of variance for root length of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	*SS*	*df*	*MS*	*obs. F*	*F (P=0.05)*
Treatment	14.312	5	2.862	1.708	2.7729
Error	30.158	18	1.675		
Total	44.470	23			

Analysis of variance for shoot length of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	*SS*	*df*	*MS*	*obs. F*	*F (P=0.05)*
Treatment	5.186	5	1.037	0.292	2.7729
Error	63.854	18	3.547		
Total	69.041	23			

Analysis of variance for wet weight of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	0.304	5	0.061	1.719	2.7729
Error	0.637	18	0.035		
Total	0.941	23			

Analysis of variance for dry weight of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	0.002	5	0.0004	1.889	2.7729
Error	0.004	18	0.0002		
Total	0.006	23			

Analysis of variance for molybdenum of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	1.705	3.4	0.501	8.301	2.6207
Error	0.966	16	0.060		
Total	2.671	19.4			

Analysis of variance for nitrate of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	12854.400	5	2570.880	1.282	2.6207
Error	48128.499	24	2005.354		
Total	60982.898	29			

Analysis of variance for total nitrogen of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	0.053	4	0.013	1.472	2.6207
Error	0.172	19	0.009		
Total	0.226	23			

Analysis of variance for total carbon of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	4.699	5	0.940	1.597	2.6207
Error	14.128	24	0.589		
Total	18.827	29			

Analysis of variance for calcium of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	0.004	4.5	0.001	0.038	2.6207
Error	0.502	21.5	0.023		
Total	0.506	26			

Analysis of variance for sodium of Caravelle cultivar muskmelon seedlings treated with a foliar application of five concentrations of Mo and an untreated control.

Source	SS	df	MS	obs. F	F (P=0.05)
Treatment	0.505	4	0.126	0.558	2.6207
Error	4.304	19	0.227		
Total	4.809	23			